REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	12/4/1995	Final Technical 8/1/1991 to 5/31/1995

4. TITLE AND SUBTITLE
Cellular and Molecular Mechanisms of High Pressure Inotropy in Cardiac Muscle.

5. FUNDING NUMBERS
G N00014-91-J-1842

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N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 Quincy Street
Arlington, VA 22217-5000

10. SPONSORING/MONITORING AGEN REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The objective of the project was to exploit isolated cardiac myocytes as a model system for investigating the effects of hydrostatic pressure on calcium regulated phenomena. This involved 5 specific aims: 1) to develop instrumentation to perform needed measurements of intracellular calcium transients in at high hydrostatic pressure and with high time-resolution; and to determine 2) if pressure acts to change resting and/or dynamic cytosolic calcium in the mammalian cardiac myocyte, 3) the role of the sarcoplasmic reticulum in pressure induced inotropy, 4) the role of sodium-potassium pump inhibition in pressure induced inotropy, 5) the role of the sodium-calcium exchanger in pressure induced inotropy. Objective 1 was met with the development of the devices and techniques developed over the course of this project. Objective 2 was met with the initial design of the pressure chamber. Further refinements were required to meet the remaining objectives, which are currently being investigated.

14. SUBJECT TERMS: Membranes, Cardiac Muscle, Contraction Force, Calcium, Inotropy, Myocyte, Pressure

15. NUMBER OF PAGES: 3

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	

Final Technical Report

Previous annual reports submitted: June 1992 for 6/3/91 to 6/1/92
June 1993 for 6/3/92 to 6/1/93
June 1994 for 6/1/93 to 6/1/94

1) Summary of original objectives

The original, broad objective of this project was to fully exploit the isolated cardiac myocyte as a model system for investigating the effects of high hydrostatic pressure on calcium regulated phenomena. To this end, specific aims *a-e* were proposed. Item *a* was partly completed in the period of our original ONR contract (N00014-88-K-0550). Pursuit of items *b-e* had to await the completion of the analytical system and the establishment of a reliable body of 1 atm data using the myocyte preparation. To date, data has been collected which addresses item *b*. We are currently in the process of performing experiments which address aims *c-e*.

a) Develop instrumentation to perform measurements of intracellular calcium transients in mammalian cardiac myocytes at high hydrostatic pressure and with high time-resolution.

This aim has been essentially completed and summarized below. The first list encompasses the aspects of this aim which were completed in the original ONR contract, while the second enumerates those completed during the current report period. The enumerated items have all been fully described in previous reports submitted to the ONR. In addition, further description of the cell pressure chamber is provided in the attached preprint of a paper which is currently in preparation for submission to Undersea and Hyperbaric Medicine.

Original contract ONR # N00014-88-K-0550:

- FURA light source designed and built.
- Line scan camera designed. Implementation started.
- Line scan memory buffer-interface designed and built.
- Microscope installed and modified for FURA measurements.
- Cell pressure chamber designed and built.
- Pressure perfusion system designed and built.
- First revision of control and analysis software designed and implemented.

Current grant ONR # N00014-91-J-1842:

- Line Scan Camera finished.
- In Bath electrodes perfected.
- Pressure chamber modified for higher pressures.
- Laminated window developed.
- Myocyte isolation apparatus built.
- Myocyte Isolation methods perfected.
- Fura loading method perfected.

- Simultaneous FURA and cell length comes on line
- Control and Analytical software completed.

In addition, it was necessary to obtain a new lens for the microscope which had better performance in the UV bandwidths used to excite the FURA dye. This lens was purchased using departmental funds.

b) Determine if pressure acts to change resting and/or dynamic cytosolic calcium in the mammalian cardiac myocyte.

1) Force frequency relationship verified in isolated myocyte at 1 atm and at pressure.

2) Force frequency relationship correlates with intracellular calcium at 1 atm and at pressure.

The following is repeated verbatim from our last annual report:

We have now demonstrated that the myocyte responds to increased hydrostatic pressure (100 atm) with an increase in resting tension and an increase in the amplitude of the phasic contraction, just as in the intact myocardium. Associated with these changes in mechanical performance is an increase in the diastolic level of cytosolic calcium. Further, while the absolute amplitude of the calcium transient is not affected by pressure, peak calcium during the transient does increase due to the higher diastolic level from which the transient is initiated.

This represents the first time measurements of the cardiac calcium transient have been made at pressure.

c) Determine the role of the sarcoplasmic reticulum in pressure induced inotropy.

No progress

d) Determine the role of Sodium-Potassium pump inhibition in pressure induced inotropy.

No progress

e) Determine the role of the Sodium-Calcium exchanger in pressure induced inotropy.

No progress

2) Publications and Abstracts

Li, M., Hong, S.K., Paganelli, C.V., Hogan, P.M. Assay of inert gas contamination in studies of hydrostatic pressure effects. Undersea and Hyperbaric Medicine. 20:163-170, 1993.

Hogan, P.M., Besch, S.R. Cellular mechanisms of hydrostatic pressure induced phenomena in nerve and muscle. Abstracts - XXXII Congress of the IUPS. Abstract 76.1/0, P27, 1993.

Hogan, P.M., Besch, S.R. A Dual Wavelength Microfluorimeter for Measuring Fast Intracellular Calcium Signals. J. Microscopy Soc. of Am., 1(2):55-63, 1995.

Hogan, P.M., Besch, S.R. Vertebrate, Skeletal and Cardiac Muscle. In "Advances in Comparative and Environmental Physiology: Effects of High Pressure on Biological Systems." A.E. Macdonald, ed. Chapter 4, pp. 125-146. Springer-Verlag, Berlin, Heidelberg, New York, 1993.

Besch, S.R., Hogan, P.M. A small chamber for making optical measurements on single living cells at elevated hydrostatic pressure. In preparation. Draft copy attached.